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Significant occurrence of *Musellifer profundus* Vivier, 1974 (Gastrotricha, Chaetonotida) in the Black Sea

Nelli G. Sergeeva[1], Derya Ürkmez[2], M. Antonio Todaro[3]

1 The A.O. Kovalevsky Institute of Marine Biological Research of RAS, 2, Nakhimov av., 299011, Sevastopol, Russia. **2** Scientific and Technological Research and Application Center, Sinop University, TR57000 Sinop, Turkey. **3** Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi, 213/D, I-41125 Modena, Italy.
Corresponding author: M. Antonio Todaro, antonio.todaro@unimore.it

Abstract
Specimens of the gastrotrich genus *Musellifer* are recorded for the first time from the Black Sea. These specimens, identified as *M. profundus* Vivier, 1974, were found in sediment samples collected at various depths (22−135 m) and from waters characterized by low salinity (17.3−18.62‰) and varying levels of oxygen (2.43−299.59 μM). Three specimens were found from the Istanbul Strait (Bosphorus) outlet area of the Black Sea (Turkey) and 2 were found along the southern and southeastern shelf of the Crimean Peninsula (Russia), from the Yalta Gulf and the Feodosiya Gulf.

Key words
Benthos, biodiversity, Bosphorus, Crimea, meiofauna, Russia, Turkey.

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Introduction

Gastrotricha is a phylum of microscopic, free-living, aquatic worms, phylogenetically close to the Platyhelminthes forming the Rouphozoa within the protostomian clade Spiralia (e.g. Struk et al. 2014, Egger et al. 2015). The phylum is cosmopolitan and counts about 840 marine and freshwater species (WoRMS 2018). Marine taxa are mainly interstitial, whereas freshwater forms are principally epibenthic with some species being interstitial or planktonic (e.g. Todaro and Hummon 2008, Kånneby and Todaro 2015). In contrast to the relatively well-known fauna of the Mediterranean Sea, gastrotrichs of the Black Sea are poorly studied (Todaro et al. 2003 and reference therein). Records date back to the beginning of the second half of the past century and come from Valkanov (1937, 1957) and Rudescu (1966, 1967, 1968). These authors studied respectively the gastrotrich fauna of the Bulgarian and Romanian coasts of the Black Sea. In summarizing their works, Todaro et al. (2003) provided a list of 29 species in 17 genera and seven families. Twenty-nine species have been recorded from Romania and 11 species from Bulgaria. All records come from samples collected in the coastal zone, from the littoral to 2 m water depth. Herein, we report on some gastrotrich specimens found in deeper waters during a survey concerning the meiofauna of the Black Sea. These gastrotrichs belong to the genus *Musellifer*, which was previously unknown from this basin.

Within Gastrotricha, the genus *Musellifer* is important from both phylogenetic and ecological perspectives; the genus is the earliest branch of the Chaetonotida Pau-

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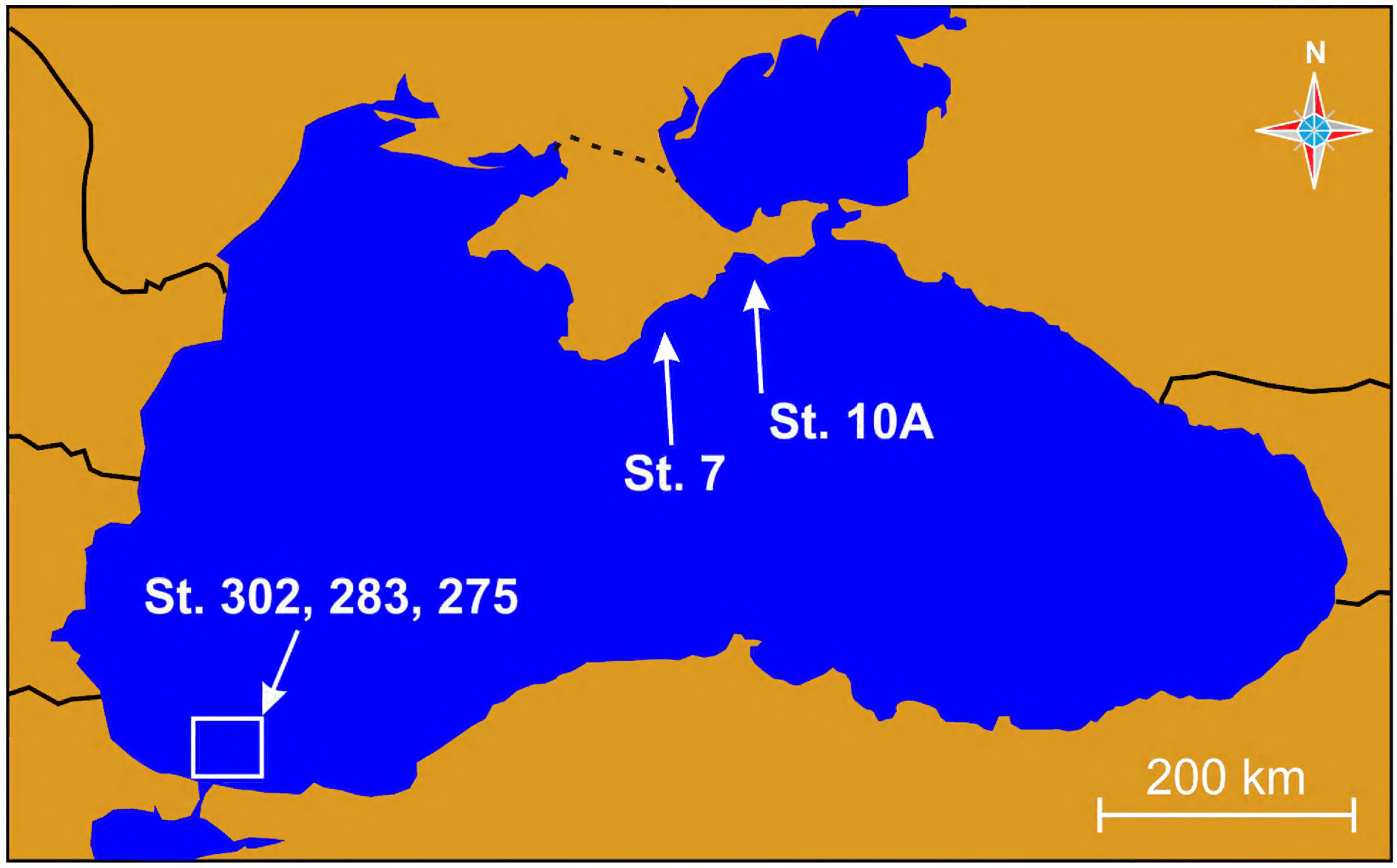

Figure 1. Sampling stations in the Black Sea where specimens *of Musellifer profundus* were found. Stations 302, 283 and 275 are located at different depths near the outlet of the Istanbul Strait (Bosphorus) area of the Black Sea (Turkey); stations 7 and 10A, were located along the south and southeastern coast of the Crimea peninsula (Russia), at the Yalta Gulf and Feodosiya Gulf, respectively.

citubulatina clade, which includes about two-thirds of all known gastrotrichs (e.g. Leasi and Todaro 2008, Kånneby et al. 2013, 2014), and the genus includes the only genuine marine species found in muddy sediments, as all the other marine species live interstitially within sandy habitats (e.g. Leasi and Todaro 2010).

Methods

The study is part of a broader research program aimed at investigating the abundance of meiobenthic taxa along an oxygen gradient. This oxygen gradient occurs where the Black Sea brackish water mass interact with the saline richer Mediterranean waters, creating a special ecological system characterized by a rapid transition from normoxia to hypoxia to anoxia water conditions (EU 7th FP project HYPOX, EC Grant 226213; see also Sergeeva and Zaika 2013, Sergeeva et al. 2013).

Eighteen stations were sampled along a transect of oxygen conditions near the Istanbul Strait (Bosphorus) at water depths of 80–300 m (Fig.1). Samples were collected in April 2010 from on board a 15/1 cruise of the RV "Maria S. Merian" (Germany) using a TV multi-corer that sampled to 5 or 10 cm depth in the substrate. Sediment cores (9.6 cm diameter) were sectioned into 1 cm layers and immediately preserved in 75% ethanol.

Oxygen was measured with Clark-type microsensors (Revsbech and Ward, 1983) on retrieved cores in the sediment and the overlying water. The microsensors were mounted on a motor-driven micromanipulator and data acquisition was performed with a DAQ-PAD 6015 and a computer. Salinity of the overlaying water was measured with a hand-held refractometer.

Additional samples were obtained within the national programs of NASU and RAS in 2011–2017, which aimed at studying the meiobenthos from the Crimean shelf area of the Black Sea. In this case, sampling was carried out in August 2011 during the 70th cruise of the RV "Professor Vodyanitsky" along the south and southeastern shelf of the Crimean peninsula (Fig. 1). Sediment cores for the study of meiofauna were obtained either by subcoring the sediment collected by an "Ocean-25" bottom grab or directly by scuba diving. A cylindrical corer of 4.8 cm inner diameter was used in both cases. Soon after sampling, each sediment core was transferred to a plastic jar and fixed using a 75% ethanol solution.

In the laboratory, all sampled sediments were washed through two staked sieves with mesh size of 1 mm and 63 μm, respectively. The fraction retained on the 63 μm mesh sieve was transferred to a plastic jar and stained with Rose Bengal for at least 24 hours. Subsequently, the meiofaunal specimens were identified to major taxa and sorted under a binocular microscope. Single gastrotrich specimens were picked out with a micro-pipette, mounted on glass slides in glycerin jelly and the coverslip sealed with lacquer. A preliminary morphological examination was carried out with bright field optics under an Olympus CX41; final survey was carried out with Nomarski differential interference contrast optics using a Nikon Eclipse 90i microscope. During observation, specimens

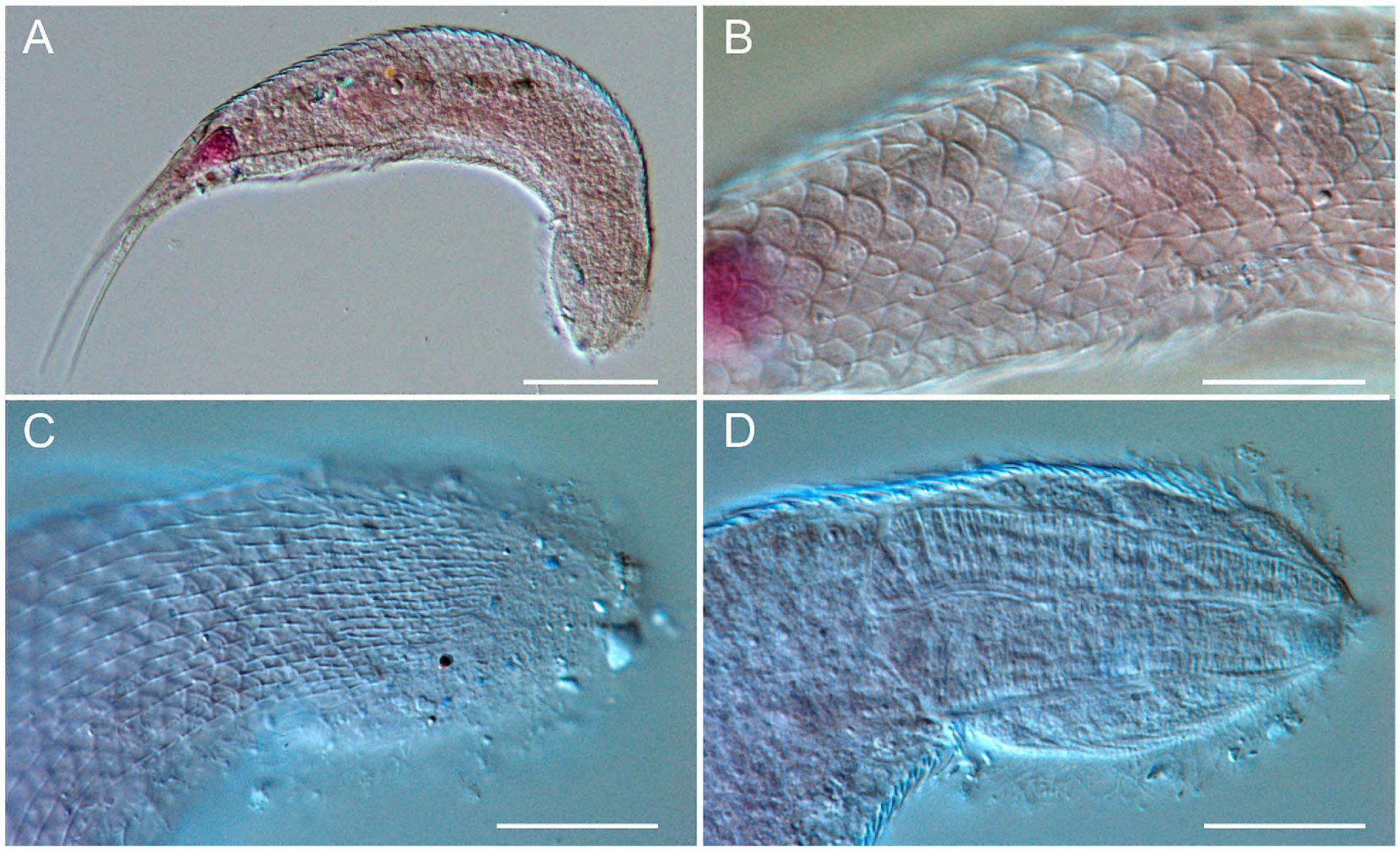

Figure 2. *Musellifer profundus* from the Black Sea. **A.** Habitus, lateral view. **B.** Posterior trunk region showing the cuticular covering. **C.** Anterior region showing the cuticular covering and ciliary arear surrounding the head. **D.** anterior region different focal plane showing the pharynx. Differential interference contrast (DIC) photomicrographs. Scale bars: **A** = 50 µm, **B–D** = 20 µm.

were photographed with a DS-5M Nikon digital camera and measured using the Nikon ACT-2U software v. 1.4.

Results

Phylum Gastrotricha Metschnikoff, 1865
Order Chaetonotida Remane, 1925 [Rao and Clausen, 1970]
Family Muselliferidae Leasi & Todaro, 2008
Genus *Musellifer* Hummon, 1969

Musellifer profundus Vivier, 1974

Material examined. Specimen 1, Black Sea: Turkey: Istanbul: Bosphorus outer area, station 302, 75/1 of R/V "Maria S. Merian" (41°24.53′ N, 029°10.56′ E), 7 April 2010, 93 m depth, in the top 1cm layer, made up of silty, normoxic sediment; in near bottom water the salinity was 18.62 ‰ and the oxygen content was 299.59 µM (IMBR RAS *microscope* slide n. B.121.Gs.v.1, 1 adult specimen, slightly curved positioned on its dorsolateral side). Specimen 2, Black Sea: Turkey: Istanbul: Bosphorus outer area, station 275, 75/1 of R/V "Maria S. Merian" (41°28.78′ N, 029°14.91′ E), 7 April 2010, 135 m depth, in the top 1cm layer, made up of muddy, hypoxic sediment; in near bottom water the salinity was 18.62 ‰ and the oxygen content was 2.43 µM (IMBR RAS *microscope* slide n. B.122.Gs.v.2, 1 subadult specimen, slightly curved, positioned on its ventrolateral side; one of the two caudal adhesive tubes appears broken). Specimen 3, Black Sea: Turkey: Istanbul: Bosphorus outer area, station 283, 75/1 of R/V "Maria S. Merian" (41°28.54′ N, 029°14.63′ E), 7 April 2010, 117 m depth, in the top 1cm layer, made up of compact brown, muddy, normoxic sediment; in near-bottom water the salinity was 18.62 ‰ and the oxygen content was 299.59 µM (IMBR RAS *microscope* slide n. B.123.Gs.v.3, 1 adult specimen positioned on its dorsal side, showing a slightly curved neck/head region, and one of the adhesive tubes bent and crossing the other). Specimen 4, Black Sea: Russia: Crimea: Yalta Gulf , station 7, 70 of R/V "Professor Vodyanitsky" (44°29.58′ N, 034°11.67′ E), 19 August 2011, 28 m depth, in muddy, normoxic sediment; in near-bottom water the salinity was 17.3‰ (IMBR RAS *microscope* slide n. B.124.Gs.v.4, 1 adult specimen slightly curved, positioned on its dorsolateral side, showing the adhesive tubes crossing). Specimen 5, Black Sea: Russia: Crimea: Feodosiya Gulf, station 10A, 70 of R/V "Professor Vodyanitsky" (45°03.18′ N, 035°41.82′ E), 20 August 2011, 22 m depth, in muddy, normoxic sediment; in near-bottom water the salinity was 17.3‰ (IMBR RAS *microscope* slide n. B.125.Gs.v.5, 1 adult specimen, positioned on the lateral side, showing the distal portion of the furca cut off).

Identification. The body of all the specimens appears compact, slightly spiny, with a relatively wide head surrounded by thin, long frizzy cilia forming a dense ciliary belt; head narrows anteriorly to a muzzle; neck constriction, weak; trunk robust ending with a furcate caudum (Fig. 2A). Furca, about ⅓ of the total length having the proximal half covered by scales. Mouth is relatively small, continuing into a robust pharynx whose diameter increases from anterior to posterior (Fig. 2D). The

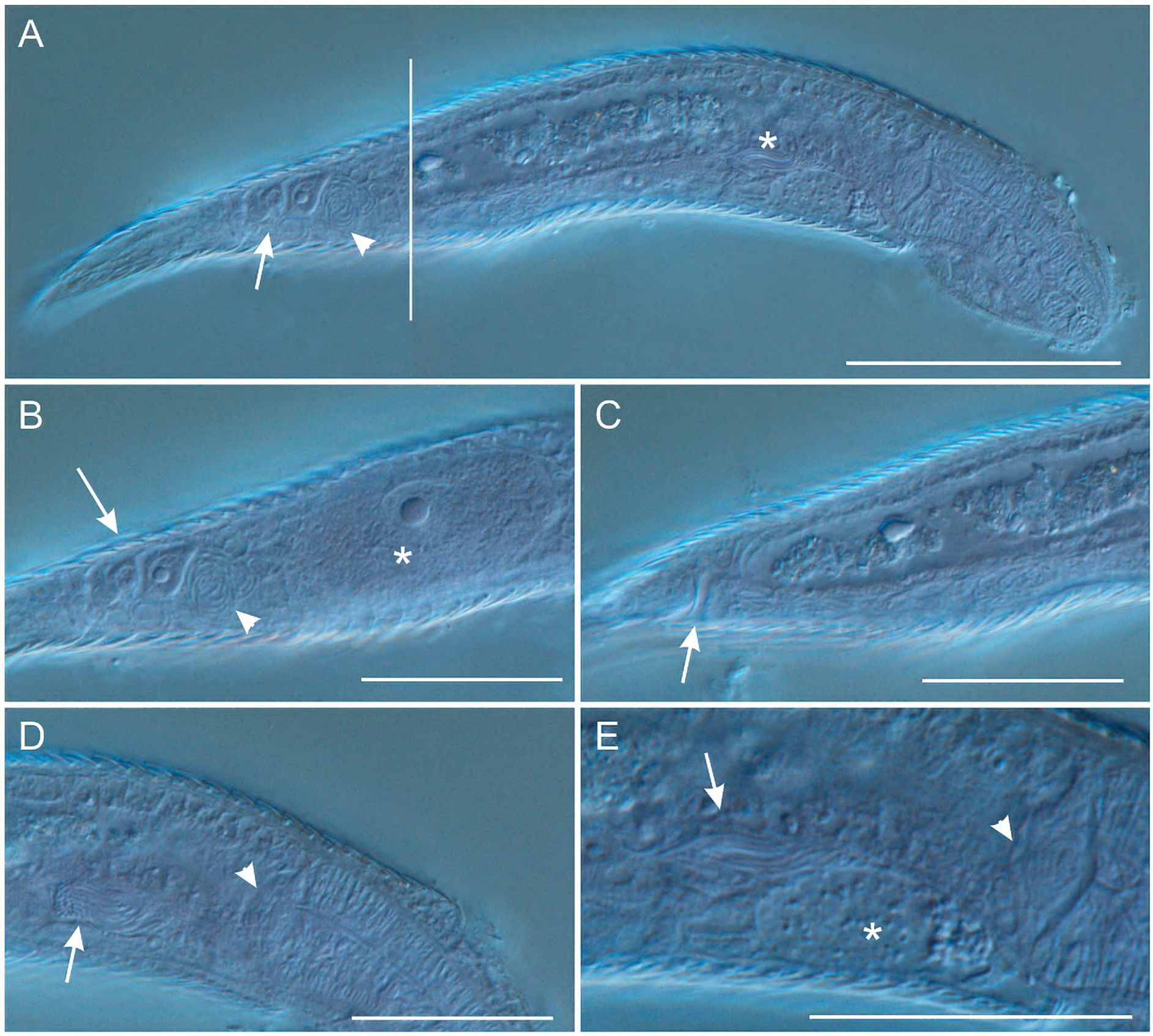

Figure 3. *Musellifer profundus* from the Black Sea. **A.** specimen in lateral view, showing the right ovary (arrow), allosperm (arrowhead) and right testis (asterisk). **B.** posterior trunk region, showing the ovary (arrow), largest oocyte (asterisk) and allosperm (arrowhead). **C.** posterior trunk region, different focal plane, showing the anus and the possible duct for the internal fertilization (arrow). **D.** anterior trunk region, showing the right testis (arrow) and pharyngo-intestinal junction (arrowhead). **E.** anterior trunk region at a different focal plane, showing the inside portion of the right testis (arrow), a glandular organ (asterisk) and pharyngo-intestinal junction (arrowhead). Differential interference contrast (DIC) photomicrographs. Scale bars: **A** = 50 µm, **B–E** = 30 µm.

intestine is straight, slightly wider at its middle, and ends with a ventral anus (Fig. 3C). The body is completely enveloped by spined scales, except for the head's anterior ciliary areas and the distal half end of the furcal branches (Fig. 2B, C). Generally, scales overlap strongly, except for the end of the proximal half of the furca, where the anterior margin of scales is barely hidden by the posterior margin of the preceding elements. The shape of the scales resemble a pentagonal arrowhead with a broadly rounded tip and spiny posterior corners. Scales of largest size, wider than long (up to 6.5 × 6 µm), cover the dorsal side of the trunk whereas relatively smaller scales, longer then wide (down to 2 × 3 µm), cover the ventral side and the furcal branches; each scale bear a simple, delicate spine, which emerges as the continuation of a poorly-defined median keel. The hermaphroditic reproductive apparatus includes paired ovaries and testes. Ovaries are located in the posterior trunk region with oocytes maturing in a caudo-cephalic direction and the largest egg located at about mid trunk, dorsal to the intestine (Fig. 3A, B). Testes are located along the anterior third of the intestine; they are rather small and pear shaped; short sperm ducts; they extend ventrolaterally and to the fore, terminating medially in a common, ovoid, glandular organ (Fig. 3A, D, E). Additional spermatozoa (allosperm), are visible in the posterior trunk region interspersed between the ovaries (Fig. 3A, B).

Discussion

The general body shape, the head narrowing anteriorly to a muzzle, and the hermaphroditic reproductive apparatus, identify the studied specimens as members of the genus *Musellifer* Hummon, 1969 (Chaetonotida, Paucitubu-

latina, Muselliferidae). Currently, *Musellifer* includes 5 sublittoral species; 4 species have been described from the Atlantic Ocean and connected seas, and 1 species, *M. sublitoralis* Hummon, 1969, has been recorded from the Pacific coast of the USA (Washington State and Alaska), and from Japan (Hummon 2010). Of the 4 species from the Atlantic and connected seas, 3 are interstitial taxa that inhabit sandy substrata: *M. delamarei* (Renaud-Mornant, 1968), *M. reichardti* Kånneby, Atherton & Hochberg, 2014 and *M. tridentatus* Kånneby, Atherton & Hochberg, 2014. *Musellifer delamarei*, originally described from the island of Ischia (Italy), has been subsequently recorded from several other Italian localities (Todaro et al. 2001). This species has recently been found outside the Mediterranean Sea at Lanzarote (Canary Islands) (Todaro et al. 2017). *Musellifer reichardti* and *M. tridentatus* have been described from a single site each, in Florida (USA) and Tobago, respectively (Kånneby et al. 2014). The fourth Atlantic species, *M. profundus* Vivier, 1974, is an epibenthic species inhabiting muddy substrata; its occurrence has been recorded throughout the Mediterranean Sea and possibly also in the North and in the Baltic seas (Leasi and Todaro 2010 and references therein). Additional species associated to muddy substrata have been documented from other regions of the world but they remain undescribed (e.g. Hummon 2010, M.A. Todaro unpublished).

The general morphometric characteristics of the present specimens (Table 1, Figs 2, 3), and the microhabitat in which they were found (muddy substrata), both indicate that the Black Sea gastrotrichs belong to *M. profundus*, a species which is widespread in the nearby Mediterranean Sea. The layout of the reproductive system, which includes ovaries and allosperm in the posterior trunk region, and compact testes, associated with an ovoidal glandular organ, in the anterior trunk region (Figs 2, 3) reinforce the identification. The specimens were found in samples collected at 5 stations, 3 in the Bosphorus area (Turkey) and 2 along the south and southeastern shelf of the Crimean peninsula (Russia); in each station only a single specimen was found. These data testify to the widespread occurrence of the species within the basin, although few in numbers. Physical and chemical characteristics of the water at the sampled stations varied to a limited extent (see above), but the finding of species in low salinity waters is of particular significance, considering that in the Mediterranean Sea the species has been recorded from normo-haline waters (35–38‰). The vouchered euryhaline nature of the species may also justify the occurrence of the species in the Baltic Sea (Leasi and Todaro 2010). Future studies that compare the Gastrotricha of the Mediterranean and Black Sea should consider the use of appropriate genetic markers (e.g. COI) to assess haplotype diversity in what may be 2 distinct populations or perhaps a geographically large but low abundant single population (e.g. Kieneke et al. 2012).

In this work, we have focused only on representatives of the genus *Musellifer*, although other gastrotrich species belonging to other genera have been found in our samples. While morphological artifacts, due to fixation, prevented us from a complete identification of these animals, their preliminary survey disclosed an unsuspected high diversity of gastrotrichs within the deep waters of the Black Sea Basin (to 150 m deep), and calls for future studies, ideally on living specimens using high-resolution optical equipment.

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Authors' Contributions

NGS collected the animal, made the slides and performed preliminary faunistic assessment. MAT performed the DIC morphological survey and identified the specimens. MAT, NGS, and DÜ wrote the text.

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